

Tumour necrosis factor α in severe congestive cardiac failure

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Abstract

Objective—To examine the concentration of circulating tumour necrosis factor α (TNF α) in patients with severe congestive heart failure (New York Heart Association class IV) during one year and to correlate changes in this cytokine with changes in plasma noradrenaline, plasma renin activity, and weight.

Design—A prospective study of the role of TNF α in severe chronic heart failure. Blood samples were collected at intervals of three months.

Setting—Medical research centre of a teaching hospital.

Patients—16 patients with chronic stable severe heart failure.

Interventions—Vasodilator treatment with captopril or flosequinan.

Main outcome measures—Changes in TNF α and the correlation with changes in plasma noradrenaline, plasma renin activity, and weight during optimal medical treatment for one year.

Results—The mean concentration of TNF α was greater than the upper 95% confidence interval for healthy controls throughout the year of the study but there was considerable between and within patient variation. No correlation was seen between TNF α and plasma noradrenaline, plasma renin activity, or weight.

Conclusions—The stimulus resulting in enhanced plasma concentrations of TNF α in congestive heart failure remains unclear and concentrations at any particular time were not prognostic.

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Various explanations have been proposed for the weight loss that is often noted in patients with severe heart failure including reduced appetite and caloric intake,^{1,2} malabsorption,³ and impaired delivery of oxygen to peripheral tissues.⁴ Resting energy expenditure in chronic heart failure is noticeably increased with increased oxygen consumption, ventilation, and heart rate compared with controls.⁵ The increased concentrations of circulating catecholamines have been implicated in mediating the catabolism of severe heart failure and the hypothesis is proposed that the resultant accelerated breakdown of adipose tissue and muscle is a consequence of the increased metabolic demands of the heart and respira-

tory muscles.^{1,4,6} Weight loss may also result from the enhanced secretion of an endogenous hormone with antianabolic or catabolic properties that has not yet been identified.

Tumour necrosis factor α (TNF α) is a cytokine secreted by macrophages and monocytes in response to a variety of stimuli and concentrations were found to be high in patients with heart failure, in association with noticeable activation of the renin angiotensin system.⁷ There was, however, a wide variation in TNF α between patients and in many it was not detected. The effects of TNF α in chronic diseases may relate more to the duration of exposure and therefore this study was undertaken to assess serial changes in TNF α during one year and the relations between the concentration of this cytokine and plasma noradrenaline, plasma renin activity, and weight in a group of patients with severe congestive heart failure who were receiving optimal medical treatment.

Patients and methods

Sixteen patients (mean aged 56 SEM 4.2 years) with severe heart failure (New York Heart Association class IV) entered the study. The mean cardiothoracic ratio was 59% (0.01%). The aetiology of the heart failure was coronary artery disease in nine patients and idiopathic dilated cardiomyopathy in seven. At the start of the study the diuretic dose was titrated to eliminate peripheral oedema before the patient received vasodilator treatment (captopril or flosequinan). The diuretic dose was adjusted as necessary during the study but the dose of vasodilator was kept constant if possible. None of the patients had any evidence of inflammatory or neoplastic disease.

After 20 minutes supine rest, venous blood was taken for measurement of packed cell volume, serum electrolytes, urea, and creatinine concentrations, plasma renin activity, and noradrenaline and TNF α concentrations. Blood for hormone analysis was placed on ice, separated immediately, and stored at -70°C until assay in batches. Samples were taken before vasodilator treatment, and after three, six, nine, and 12 months of vasodilator treatment.

ASSAYS

Plasma renin activity was measured by radioimmunoassay and noradrenaline by high performance liquid chromatography. The TNF α was measured by enzyme linked

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Figure 1 Plasma tumour necrosis factor α (TNF α) in 16 patients with severe heart failure studied prospectively for one year. Median values are represented by horizontal lines. D indicates patients who died during the study.

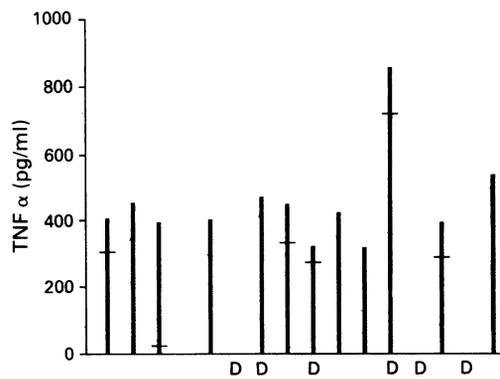


Figure 2 Plasma noradrenaline and tumour necrosis factor α (TNF α) during one year in 16 patients with severe heart failure ($r = 0.36$).

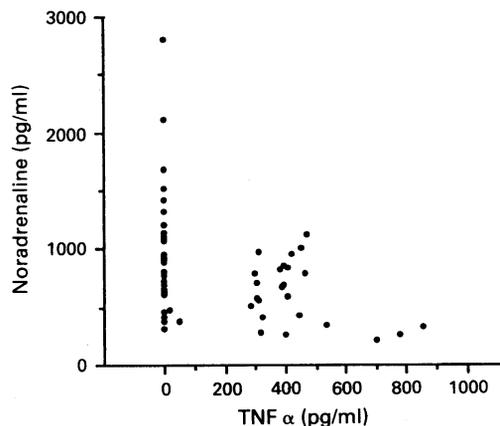
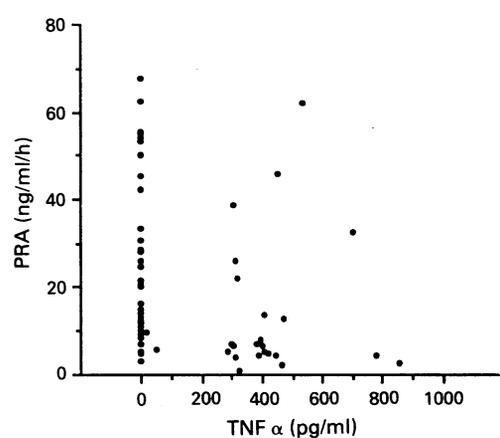


Figure 3 Plasma renin activity (PRA) and tumour necrosis factor α (TNF α) during one year in 16 patients with severe heart failure ($r = 0.23$).



immunoabsorbent assay (ELISA).⁸ Briefly a microlitre plate was coated with monoclonal antibody to TNF α at 4°C overnight. The plates were blocked with 2% bovine serum albumin in phosphate buffered saline (PBS) and either standards or samples diluted in PBS containing 0.1% gelatin and 0.05% tween were added. After overnight incubation

50 μ l/well of rabbit anti-TNF α was allowed to act for two hours, the plates were washed, antirabbit IgG antibody was added. After a further hour, peroxidase substrate mixture (100 μ l/well) was added and allowed to act for 30 minutes and the reaction was then stopped by the addition of 50 μ l 3M sodium hydroxide into each well. Absorbance was read at 455.5 nm. Each plate consisted of eight wells without TNF α (buffer only) and a standard curve for TNF α of 62.5 to 2000 pg/ml in duplicate. The concentration of TNF α in each sample was calculated from a regression line derived from the standard curve. The sensitivity of the assay was 28.25 pg/ml. The upper limit for TNF α in healthy controls in our laboratory is 65 pg/ml (upper 95% confidence interval (95% CI). All results are expressed as mean (SEM).

STATISTICAL METHODS

The two tailed Wilcoxon paired rank sum test was used for all statistical analyses.

Results

Plasma TNF α was increased in the group of patients with severe heart failure at entry to the study (173.5 (63.5) (mean SEM)) pg/ml, range 0–855 pg/ml, $n = 16$ and after one year (142.2 (58.75) pg/ml, range 0–405 pg/ml, $n = 10$; $p = 0.844$). There was considerable variation in individual patients during the year of the study, and in all of the 16 patients TNF α was not detected on at least one occasion (fig 1). In two of the six patients who died during the study circulating TNF α was never detected.

There was no suggestion of a direct relation between plasma TNF α and either plasma noradrenaline (fig 2) or plasma renin activity (fig 3). No correlation was found between TNF α and noradrenaline either at entry into the study ($r^2 = 0.03$, $p = 0.52$, $n = 16$) or in the survivors at one year ($r^2 = 0.37$, $p = 0.06$, $n = 10$). The same was found between TNF α and plasma renin activity at entry ($r^2 = 0.17$, $p = 0.11$, $n = 16$) and at one year ($r^2 = 0.15$, $p = 0.27$, $n = 10$). The mean (SEM) plasma noradrenaline of the group did not change significantly during the study after the introduction of vasodilator treatment (751.6 (111.6) pg/ml at entry, 793.9 (108) pg/ml at one year). The mean plasma renin activity, however, did increase from 10.4 (3.4) ng/ml/h at entry to 27.4 (7.4) ng/ml/h at one year ($p = 0.06$).

No significant changes were seen in mean sodium, potassium, urea, and creatinine in serum although there was a trend for creatinine to rise during the study (table). There was a significant fall in packed all volume but the decrease in haemoglobin concentration was not significant. The dose of frusemide required by these patients with severe heart failure increased significantly during the course of the study (table).

Little change occurred in the mean weight of the group. The mean (SEM) weight change from entry to the last recorded weight

Biochemical, diuretic, and weight characteristics (mean (SEM)) of patients with severe heart failure

| | At entry ($n = 16$) | One year later ($n = 10$) | p Value |
|---------------------------|-----------------------|-----------------------------|-----------|
| Serum sodium (mmol/l) | 138.75 (1.17) | 137.60 (0.64) | 0.11 |
| Serum potassium (mmol/l) | 4.34 (0.09) | 4.26 (0.11) | 0.81 |
| Urea (mmol/l) | 9.04 (1.14) | 9.01 (1.21) | 0.42 |
| Creatinine (μ mol/l) | 129.0 (6.41) | 142.18 (11.42) | 0.64 |
| Haemoglobin (mg/dl) | 15.37 (0.48) | 14.69 (0.24) | 0.22 |
| Packed cell volume (%) | 48.25 (1.39) | 44.81 (0.48) | 0.012 |
| Frusemide dose (mg/day) | 90.0 (4.47) | 130.6 (11.68) | 0.009 |
| Weight (kg) | 80.55 (4.01) | 80.24 (6.11) | 0.98 |

was -1.82 (0.9) kg, with a maximum fall of 5.35% of the initial weight (table 1).

Discussion

In our study of patients with severe heart failure the mean concentration of plasma immunoreactive TNF α was increased during the year of the study. There was, however, considerable between and within patient variation and no correlation was found between the concentration of circulating TNF α and mortality (one year mortality was 40%). All patients were treated with vasodilators throughout the study and the dose of diuretic adjusted to eliminate oedema if necessary. The dose of diuretic required increased significantly during the study and may account for some of the increase in plasma renin activity. Body mass indices were not measured but the mean weight of the group did not change. There was no significant relation between TNF α and noradrenaline or TNF α and plasma renin activity.

Measurements of TNF α were made by an ELISA that detects biologically inactive fragments of TNF α . Both antibodies used in our assay were, however, neutralising to the cytotoxic activity of TNF α and we have previously shown a good relation between the ELISA used in this study and the WEHI 164 cell cytotoxicity bioassay for TNF α for plasma specimens and recombinant TNF α .⁹

Monocytes and macrophages synthesise and secrete TNF α in response to a number of stimuli including bacterial endotoxaemia in both animals and humans.¹⁰ It is a mediator of septic shock and multiple organ failure and its effects can be abolished by passive immunisation with antibodies to recombinant TNF α . The half life of TNF α in human plasma is a few minutes.¹⁰ Infusion of a bolus of endotoxin in normal subjects resulted in high concentrations of TNF α (measured by ELISA) at two hours. These had returned to normal after four hours.¹¹ Metabolic effects, such as an increase in temperature, heart rate, adrenocorticotrophic hormone, and noradrenaline were seen after two hours and persisted for up to six hours despite the return of TNF α concentrations to normal. A single injection of recombinant TNF α in humans resulted in a pyogenic response with associated tachycardia, increased oxygen consumption and carbon dioxide production, and an increase in protein metabolism that mimicked the acute dose of endotoxin.¹² Some of these changes are similar to those seen in severe heart failure and it has therefore been postulated that TNF α may be a possible mediator of at least some of the metabolic responses of cardiac failure. A significant increase in circulating catecholamines after the injection of cachectin has been shown in dogs, but not in humans.¹³ High concentrations are of prognostic relevance in acute gram negative sepsis and cerebral malaria.¹⁴ Increased concentrations TNF α have also been found in a number of diseases associated with cachexia

including human immunodeficiency virus related disease, trypanosomiasis infestation, and in patients with cystic fibrosis chronically infected with *Pseudomonas aeruginosa*.^{6,14} There is, however, no definitive proof that TNF α has an important pathogenic role in the development of cachexia and anorexia. In these conditions as there is likely to be a complex interaction among a number of cytokines that have been shown to have both metabolic and anorectic effects.

Recent studies on patients with heart failure have suggested that the relation between the weight loss and concentration of TNF α at any particular point in the natural history of the condition may be causally linked. The evidence from these cross sectional studies should be interpreted with caution. In the study by McMurray *et al*, TNF α was not detected in 90% of their non-cachectic patients with severe heart failure or in 44% of patients with cachexia.¹⁵ Levine *et al* found increased concentrations of TNF α in their group of patients with high renin concentrations.⁷ Again there was a wide variation and in many of these patients circulating TNF α was not detected. The wide variation in the concentration of circulating TNF α in these studies and in our prospective study warrants further investigation before the suggestion that the therapeutic manipulation of TNF α in severe heart failure might be beneficial. The role of this cytokine in the natural history of chronic heart failure remains unknown.

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